PATENT
Attorney Docket No. 010091-001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	
Richard SCHLEGEL et al	
Serial No. 08/216,506	Group Art Unit: 1813
Filed: March 22, 1994	Examiner: A. Caputa
For: PAPILLOMAVIRUS VACCINE	

DECLARATION OF C. RICHARD SCHLEGEL AND A. BENNETT JENSON UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

We, C. Richard Schlegel and A. Bennett Jenson, hereby declare and state as follows:

(1) that C. Richard Schlegel resides as 3 Elmwood Park, Rockville, Maryland 20850 and A. Bennett Jenson resides at 14220 Briarwood Terrace, Rockville, Maryland 20853;

(2) that C. Richard Schlegel and A. Bennett Jenson are the co-inventors of the subject application;

(3) that we have reviewed the most recent Office Action issued by Examiner Caputa on September 20, 1995, including the new references cited therein. More specifically, we considered Browne et al, General Virology 69:1263-1273 (1988) hereinafter Browne et al (Id.); Minson, U.S. Patent No. 5,045,447, hereinafter Minson (Id.); Danos et al, U.S. Patent No. 4,551,270; and Carter et al, Virology 182:513-521 (1991). It is our understanding that the Examiner has concluded that these references separately or in combination would suggest compositions containing

conformationally correct human papillomavirus (HPV) L1 proteins and their usage for providing immunity against HPV infection. It is our further understanding that the Examiner has concluded that the recombinant methods of producing HPV L1 proteins taught by Carter et al (Id.), Minson et al (Id.) or Browne et al (Id.) would inherently result in conformationally correct L1 proteins that could be used as vaccines for affording immunity against HPV infection. Based on the following, the Examiner's conclusion is factually in error.

(4) With respect to Browne et al (Id.), we have carefully reviewed this reference in its entirety. This reference relates to the expression of HPV-16 L1 protein using a vaccinia expression system. However, contrary to the Examiner's conclusion, this methodology would not give rise to conformationally correct L1 proteins. This is because the authors express the same prototype HPV-16 sequence which was expressed by Zhou et al (Journal of Virology 185:251-257 (1991)). This is readily apparent based on their source of the HPV-16 DNA used for expression. In particular, it is noted that their HPV-16 L1 DNA was obtained from Dr. Zur Hausen (see page 1264 of the reference) who is a collaborator of Dr. Gissman who supplied the defective mutant HPV-16 L1 DNA which was expressed by Zhou et al (Id.). This defective mutant sequence has already been established not to give rise to conformationally correct L1 proteins. In factual support thereof, we respectfully refer the Examiner to the previously submitted § 132 declaration by Dr. Jenson submitted with the March 8, 1995 Reply.

(5) With respect to Minson (Id.), we have also carefully reviewed this reference in its entirety. It is readily apparent from our review that the patentee also expressed the HPV-16 DNA mutant sequence which was expressed by Browne et al (Id.). This is clear for example because the patentee incorporates by reference the procedures described by Browne et al (Id.) (see column 5, lines 15-17 of the patent). This is further clear because the source of the HPV-16 L1 open reading frame is Seedorf et al (Virology 145:181 (1985)). The authors in Seedorf et al (Id.) include Zur Hausen. Therefore, this reference expresses the same mutant HPV-16 L1 sequence which was expressed by Zhou et al (Id.).

(6) We have also carefully reviewed Carter et al (Id.). This reference relates to expression of several HPV L1 and L2 proteins in *Saccharomyces cerevisiae*. More particularly, the reference describes expression of HPV-16, HPV-1 and HPV-6b L1 DNA sequences in yeast. For different reasons, which are explained in detail below, none of these methods of expression would be reasonably expected to give rise to conformationally correct HPV L1 proteins.

With respect to the HPV-16 L1 sequence expressed by Carter et al (Id.), they expressed the same defective mutant HPV-16 L1 DNA expressed by Browne et al (Id.), Minson (Id.) and Zhou et al (Id.). This is clear based on their disclosed source of the HPV-16 L1 open reading frame (*Durst et al, Proc. Natl. Acad. Sci., U.S.A.* 80:3812-3815 (1983)); and Seedorf et al, Virology 145:181-185 (1985)). We note that Durst et al and Seedorf et al are in the same lab as Zur Hausen which was the source of the defective

mutant HPV-16 L1 sequence expressed by Zhou et al (Id.). Therefore, Carter et al expressed the same mutant HPV-16 L1 sequence as Zhou et al (Id.) and accordingly would have obtained L1 proteins which do not exhibit proper conformation.

With respect to the HPV-6b sequence expressed by Carter et al (Id.), they expressed a truncated HPV-6b sequence. This is apparent for example from page 515 of the reference wherein the authors state that the HPV-6b L1 sequence encodes a 461 C-terminal amino acid sequence which comprises part of the entire 500 amino acid sequence. Therefore, the HPV-6b protein expressed by Carter lacks 39 of the N-terminal amino acids of the native HPV-6b L1 protein. Also, this truncated HPV-6b L1 sequence was expressed as a fusion with a 9 amino acid sequence (MGIRARYPG). Given the fact that Carter et al (Id.) expressed a truncated sequence lacking 39 N-terminal amino acids and further because they expressed it as a fusion protein, it would have been reasonably expected that they would have obtained non-conformationally correct L1 protein. In this regard, subsequent experiments conducted by us have demonstrated that the N-terminal portion of the L1 protein is essential for proper conformation of L1 proteins. Also, because it was expressed as a fusion protein, this would also be reasonably expected to adversely affect the conformation of the resultant truncated HPV-6b L1 protein.

With respect to the HPV-1 L1 sequence expressed in Carter et al (Id.) it is noted that Carter et al cloned their HPV-1 L1 sequence using polymerase chain reaction (PCR) cloning methods. Moreover, Carter et al (Id.) took no precautions to ensure that the resultant cloned L1 sequences did not contain errors prior to

expression. For example, the authors did not sequence their HPV-1 L1 DNA to determine whether it possessed any errors introduced by PCR cloning. Moreover, it is known that PCR cloning typically gives rise to mutation. PCR cloning techniques have an inherent infidelity (because of the enzyme used for amplification) which typically gives rise to a mutation every 300 to 500 base pairs. Consequently, given that the size of the HPV-1 L1 DNA which was cloned is about 1500 nucleotides, it would be reasonably expected that their L1 sequence which would contain on average about 3 to 5 mutations. The exact mutations probably introduced by PCR cloning are impossible to assess because Carter et al did not sequence their HPV-1 L1 DNA. Moreover, it is further impossible to demonstrate by subsequent experiments the errors likely introduced because PCR is inherently unpredictable. However, given the high level of fastidiousness of the L1 protein, it would be reasonable to conclude that at least one of the mutations which was likely introduced by PCR cloning would have potentially adversely affected the conformation of the resultant protein. We further note that the effect of such probable mutations (introduced by PCR cloning) is impossible to assess from the Carter et al reference. This is because Carter et al did not conduct any experiments which would confirm proper conformation, even assuming that conformationally correct L1 proteins would have been fortuitously obtained. L1 proteins which exhibit proper conformation can only be conformed based on their reactivity with conformationally-dependent antibodies. Rather, the authors only used antibodies to linear epitopes. Conformationally-dependent antibodies are antibodies which recognize epitopes expressed on

conformationally correct L1 proteins. However, Carter et al conducted no immunological assays with conformationally-dependent antibodies. Moreover, even assuming that they had conducted such assays, it is our expert opinion that they likely would confirm that conformationally correct L1 proteins were not obtained given that PCR cloning would have been expected to give rise to about 3 to 5 mutations in the expressed HPV-1 L1 sequence. This conclusion is further supported by experiments which have been conducted by us relating to expression of COPV-L1 carboxy terminal deletion mutants. In these experiments we have used PCR techniques to clone the COPV-L1 carboxy terminal deletion mutants. We have found that PCR typically gives rise to significant mutations as determined by nucleotide sequencing.

(7) Thus, based on the foregoing, it is our expert opinion that none of Carter et al (Id.), Browne et al, or Minson, whether considered individually or in combination, would have been reasonably expected to have obtained HPV conformationally correct L1 proteins either because of the cloning techniques they used or because of the HPV L1 sequence which was expressed.

(8) The undersigned declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of

the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: _____ _____
C. Richard Schlegel

Date: _____ _____
A. Bennett Jenson